

2606470-20030303

F. S. G. S.

DIGEST
PCR FRAGMENT
AND
pMBD202020
NdeI/BamHI
LIGATE

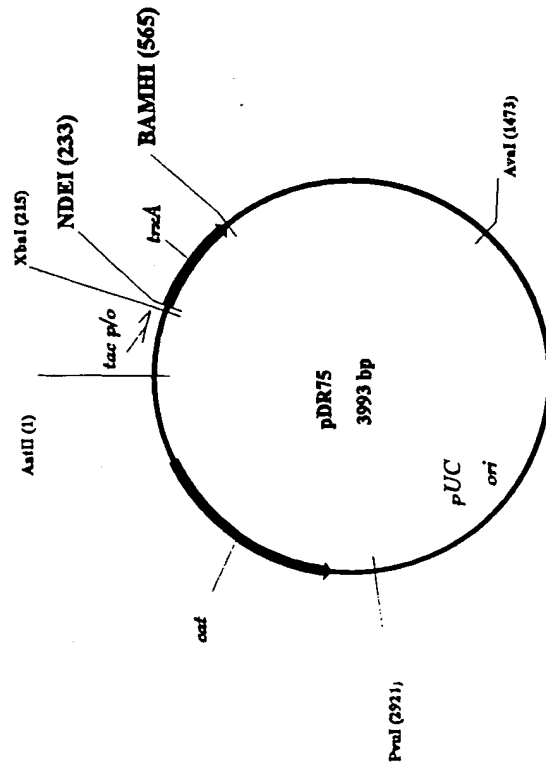
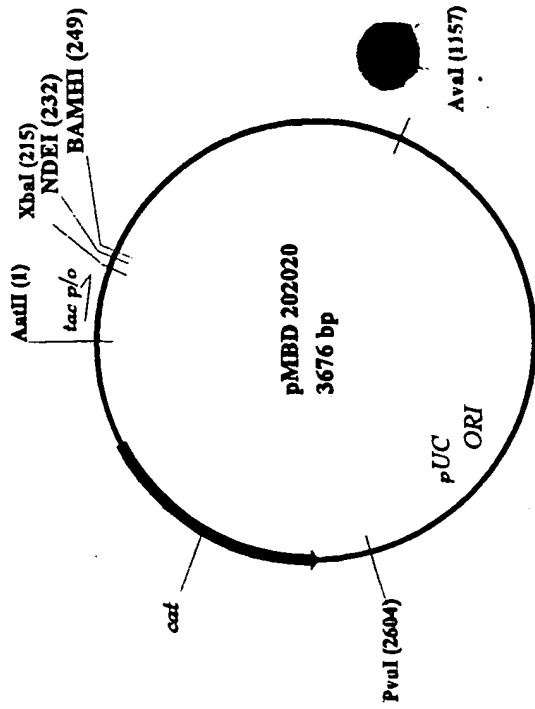
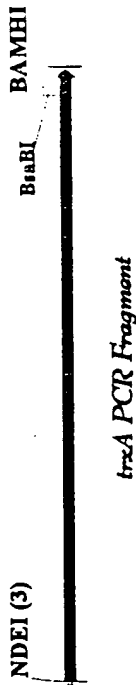
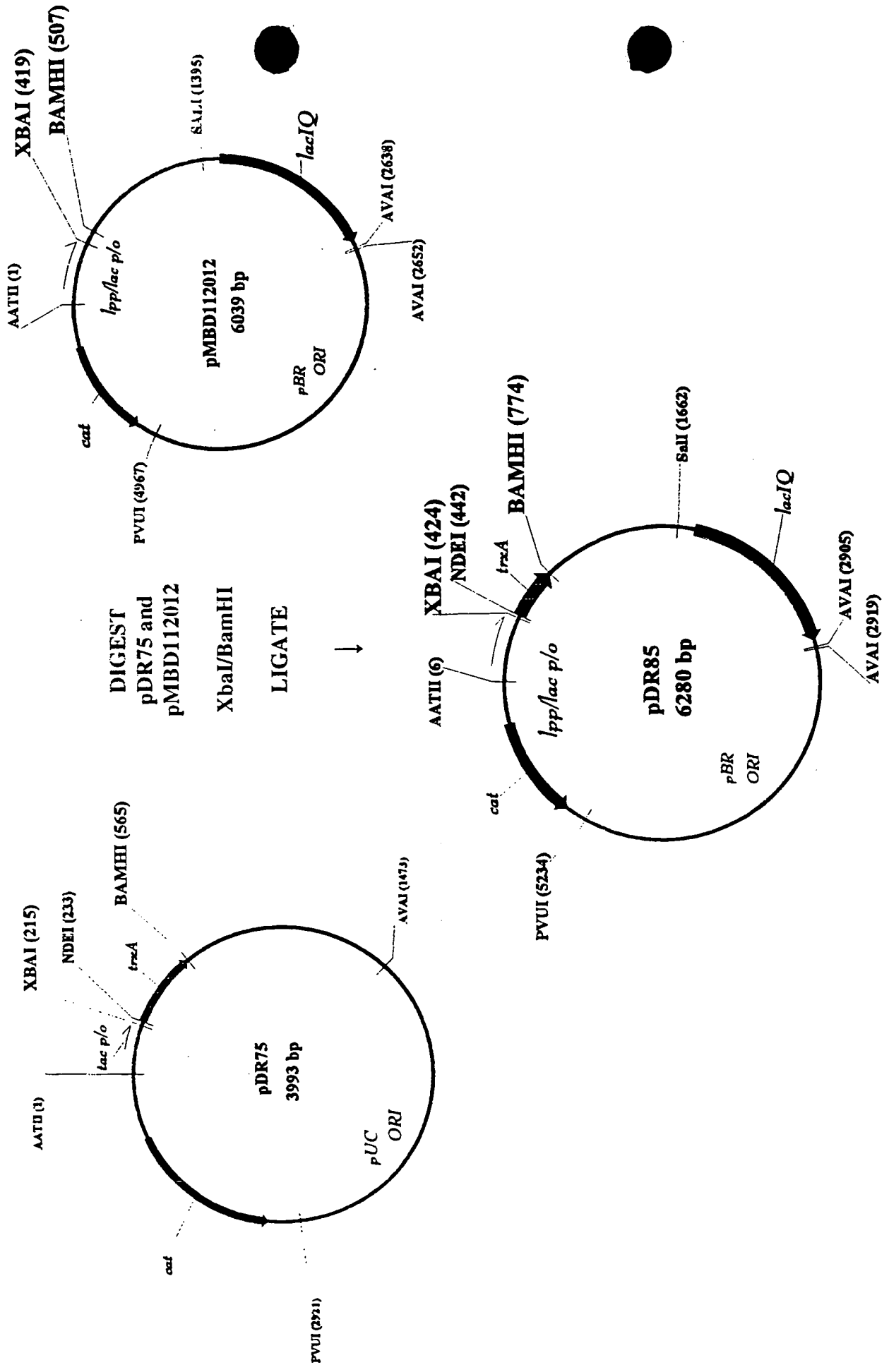
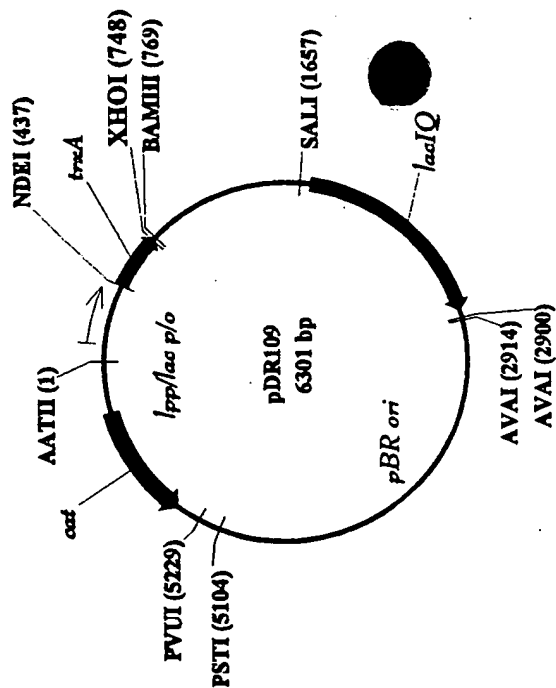
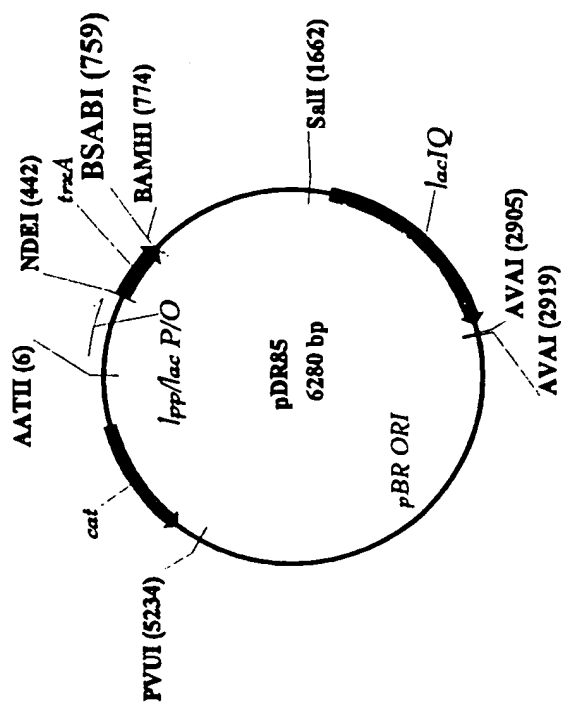


Figure 2
Schematic





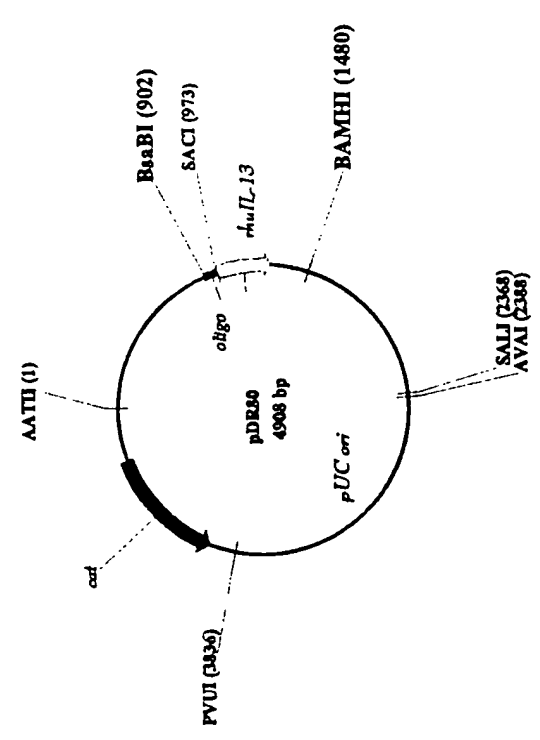
IN THE CONSTRUCTION OF pDR109 THE *trxA* GENE WAS ALTERED TO REPLACE THE BSAB1 SITE WITH A UNIQUE XHO1 RESTRICTION SITE FOR EASY SUBCLONING OF A DOWNSTREAM RECOMBINANT GENE.

Leu Asp Ala Asn Leu Ala ***
CTC GAT GCT AAT CTG GCG TAA
||| ||| ||| |||
CTC GAG GCT AAT CTG GCG TAA ***
Leu Glu Ala Asn Leu Ala ***

pDR85 Sequence

pDR109 Sequence

Figure 4

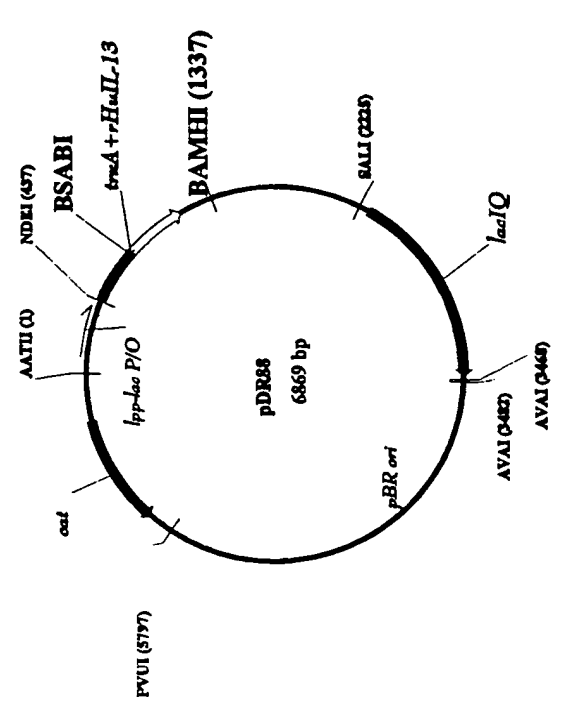
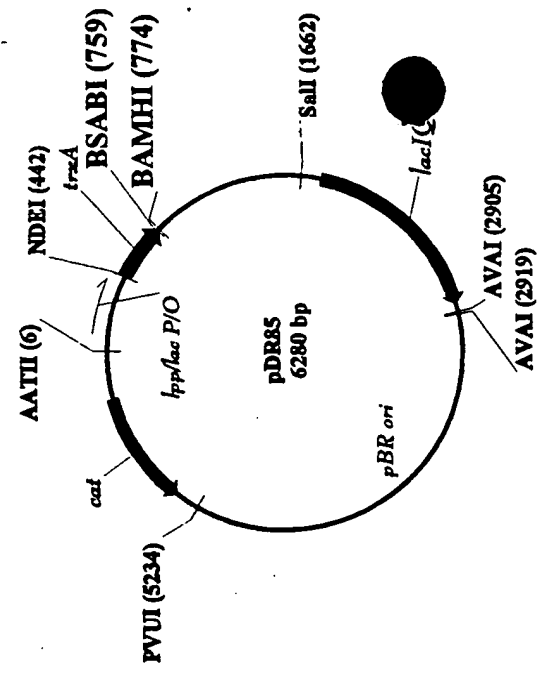


DIGEST
pDR80 & pDR85

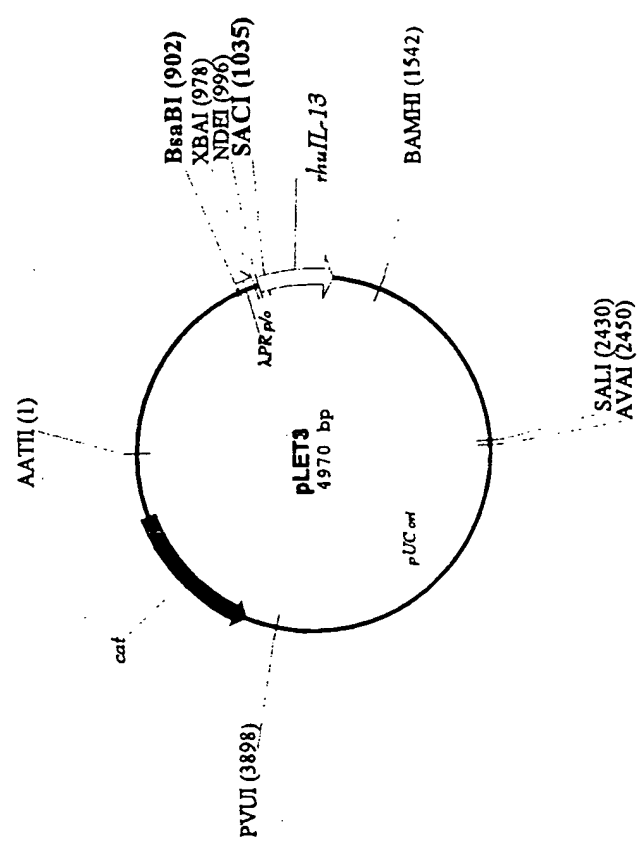
BsaBI/BamHI



LIGATE



26 Oct 93 Figure 5

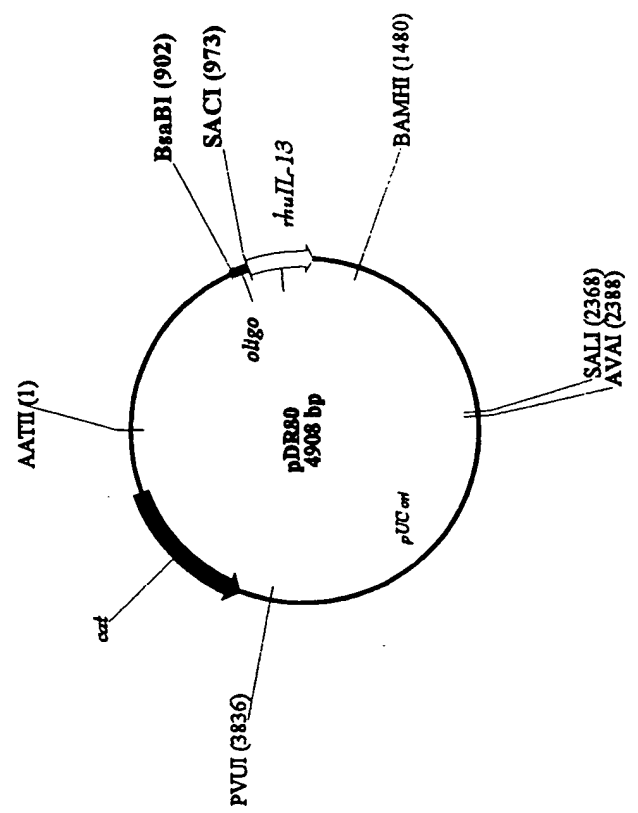


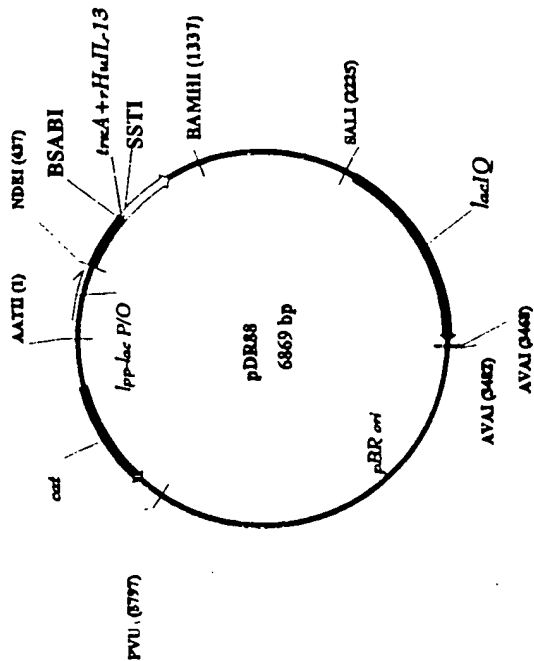
BsaBI-SacI oligo sequence			
GATAA	TAATCTGGCT	GGTCTGGTTCTGGT	GATGACGATGACAAG
trxA sequence	glyserglysergly	aspaaspasp	plys
linker region	enterokinase	cleavage	
GGTCC	TGTTCCGCCG	TCCGCTCTGC	GTGAGCTC
rhuIL-13 sequence			

Synthesize both DNA strands of the BsaBI-SacI oligo
Digest pLET3 with BsaBI-SacI



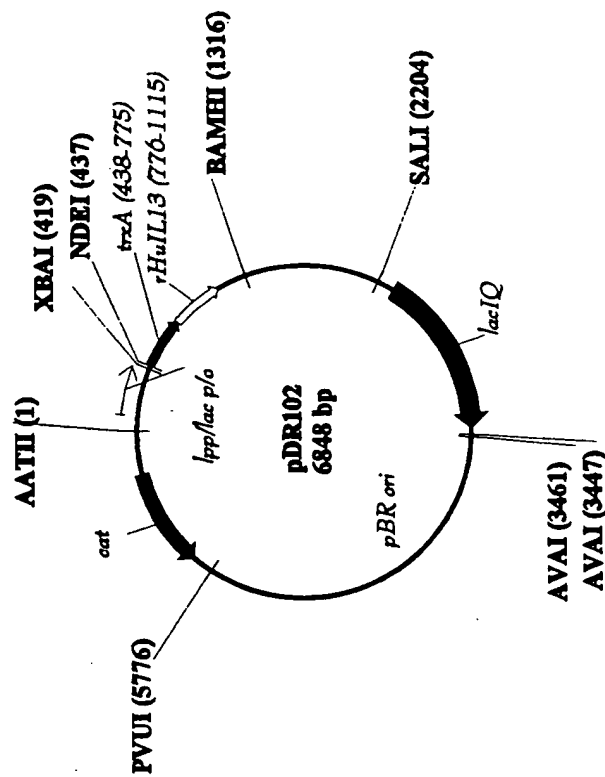
Ligate pLET3 and the Oligo





SYNTHESIZE A DOUBLE STRANDED OLIGO WITH BSABI/SSTI RESTRICTION SITES

LIGATE INTO BSAB/SSSTI DIGESTED pDR88



R.B.S.

rhull-13

Sstl

5' ATG TAC
GCG AAG GAG GCT GAT TAAATG GGT CCG GTT CCG TCT ACC GCT CTG CAG CTC

trxA

stop

Figure 7

